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FURTHER ASSESSMENT OF THE REITER PROTEIN COMPLEMENT-FIXATION (RPCF) TEST IN A ROUTINE SEROLOGICAL LABORATORY*

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At St. Thomas's Hospital we were led to try the RPCF test by the preliminary report of De Bruijn (1957), in which he showed with 116 syphilitic sera that this test was considerably more sensitive than the Wassermann reaction (WR). The technique appeared simple, the antigen was available commercially and the test was in no way difficult for the routine laboratory.

De Bruijn gave no precise details as to how he performed his test, but we understand that a Kolmer technique was used. It was therefore decided to use the Reiter antigen in a test modelled as closely as possible on our customary WR. This technique used a single 1 in 5 dilution of patients' serum which was incubated with antigen and 2·5 mhd complement at 37°C. for 1 hour with the subsequent addition of a volume of 3 per cent. sheep erythrocytes sensitized with 6 mhd haemolysin. For the RPCF test we simply substituted a volume of Reiter antigen diluted 1/80 with saline for the WR antigen and instead of being incubated for 1 hour at 37°C. the tubes were refrigerated over night and warmed to 37°C. for 15 minutes next day before the addition of the sensitized sheep cells.

It was decided to try the test in parallel with the WR and Price's precipitation reaction (PPR) on the next 1,000 consecutive sera received in the serological laboratory. The results, which have been published (Foster, Nicol, and Stone, 1958), are shown in Table I.

TABLE I

Test	WR/PPR	RPCF	Number of Cases
Either or Both	+	+	178
Either or Both	+	−	13
Both	−	+	97

The first line of Table I shows that there was agreement between WR/PPR and RPCF test in 178 positive cases.

The second shows that there were thirteen cases in which the RPCF had apparently been less sensitive or more specific than the tests for reagin. Of these thirteen patients, eight had a past history of syphilis, but in the other five there was no clinical evidence in support of the WR/PPR and they may well have been biological false positive reactions.

The most striking finding is shown in the third line; 97 cases were found in which the RPCF test was positive yet both WR and PPR were negative.

Clinical assessment of these 97 patients did not suggest that the reactions were false positives, since in 82 there was a past history of syphilis or yaws, and the remaining fifteen were coloured patients, who may have had yaws in infancy without remembering it.

Although it was not considered that the RPCF test had by any means been thoroughly assessed, it was not possible in our routine laboratory work to continue to do both the WR and the RPCF test. However, the results so far obtained suggested that there was nothing to lose by substituting the RPCF test for the WR, provided that a careful assessment was made of patients in whom there was a discrepancy between the PPR and the RPCF test.

During the first 9 months after the introduction of the RPCF test as a routine there have been 151 discrepancies between it and the PPR. Dr. Sequeira has kindly performed treponemal immobilization (TPI) tests on these sera; the results are shown in Table II.

TABLE II

Test					
PPR	+	+	−	−
RPCF	−	−	+	+
TPI	+	−	+	−
No. of Patients	2	4	116	28

* Paper read to the M.S.S.V.D. on March 20, 1959.

The first column shows two cases of treponemal disease not picked up with the RPCF test. The second comprises four cases which may be fairly considered to be biologic false positives recognized as such by the RPCF test. The third column shows 116 cases which are presumed to have past or present treponemal disease, were not diagnosed by the PPR but picked up by the RPCF test and supported by the TPI test. The last column comprises 28 cases in which the RPCF test suggests treponemal disease, but is unsupported by the TPI results.

In trying to decide whether or not these 28 results were biologic false positives, a clinical assessment was carried out. An analysis of these cases is presented in Table III.

TABLE III

History	Number of Patients
Genital Sore	3
Gonorrhoea or Urethritis	8
Syphilis	2
Yaws	2
Early Syphilis	1
Previous Penicillin Injections	18

It will be seen that in eight patients there is either a past history of a genital sore or of definite treponemal disease, while in eight others a past history of urethritis might suggest that they have had treponemal infection. The high proportion who had received injections of penicillin for one reason or another may also have resulted in the incidental cure of some previous treponemal infection. The fact that the TPI test is negative by no means excludes treponemal infection. The STS, RPCF, and TPI tests all depend upon the presence in the serum of different antibodies which neither appear nor disappear at exactly the same time. We believe that the RPCF test becomes positive early in syphilis, antedating the positive TPI, and that even in adequately treated cases it remains positive for a long time. This is illustrated by the following cases:

Two patients, male British West Indians, both with primary syphilis, *Treponema pallidum* being seen on dark-ground examination, gave the following pattern of serological reactions with successive samples of blood (Table IV).

TABLE IV

Patient	Date	RPCF	WR	PPR
1	4.2.59	+	−	
	12.2.59	+	±	1/1
	24.2.59	+	−	−
2	25.2.59	+	−	
	7.3.59	+	+	−

A third patient, also a male British West Indian, had secondary syphilis, *Treponema pallidum* being seen on dark-ground examination. His blood showed the following reactions on successive occasions (Table V).

TABLE V

Date	RPCF	WR	PPR
16.11.57	+	+	1/1
23.11.57	+	+	1/8
18. 4.58	+		−
5. 3.59	+	−	

In these three cases, a TPI test was not done; it is generally considered that the WR becomes positive before the TPI test, yet the RPCF test became positive before the WR.

Two further patients illustrate the persistence of a positive RPCF test in treated syphilis when the TPI test was negative. Both presented with the following serological reactions:

RPCF	PPR	TPI
+	−	−

The fourth case, a white female aged 48, at first denied past venereal disease, but later admitted that she had been treated for syphilis 17 years before and this was confirmed by her previous documents.

The fifth case, a white male aged 32, admitted that he had been treated for syphilis with penicillin and arsenic 13 years previously.

In contrast to these cases, we have carried out RPCF tests on 140 blood donors, of whom only two gave positive results, and in both of these the TPI was positive. Rein, Kelcec, D'Allessandro, and de Bruijn (1957) tested a large number of biological false positive reactors with the RPCF test, and had no positive results. It is therefore felt that the RPCF test is a sensitive and specific test for treponemal infection which becomes positive very early in the disease and remains positive for a long time even in treated cases.

With regard to the reproducibility of the RPCF test, we performed repeated tests on successive samples of serum, from two to five in number, in 192 cases in which the diagnosis was not absolutely straightforward and in which, therefore, relatively little antibody was probably present. The results were as follows:

Antibody	*No. of Patients*
Consistently absent	12
Consistently present	143
Variable	37

Of the 37 patients showing variable results, seventeen had positive TPI tests, which suggest that even an inconstantly positive RPCF test is quite likely to indicate past or present treponemal disease.

Dr. Sequeira has carried out RPCF tests on 132 sera in parallel with us, and his results have agreed with ours in 99 cases. In those cases in which there was a difference the TPI test supported him in fourteen and us in nineteen. The large number of discrepancies between the test used for reagin (PPR) and the Reiter antibody suggested that it might be better to use a more sensitive test for reagin, and at Dr. Sequiera's suggestion we substituted a WR with Maltaner antigen for the PPR. This allows a very convenient routine WR and RPCF test to be done on each sample of serum by setting up a rack with three tubes per test as shown in Table VI.

TABLE VI

Tube	1	2	3
1/5 Dilution Serum	+	+	+
2½ mhd Complement	+	+	+
Saline	+	–	–
Maltaner Antigen	–	+	–
RPCF Antigen	–	–	+
Reading	Control	WR	RPCF Test

The racks are refrigerated overnight, warmed to 37°C. for 15 minutes before the addition of sensitized cells, and read after a further 15 minutes.

Using the PPR and RPCF test, about 18 per cent. of sera showed a discrepancy between the tests. Using the WR and RPCF test, this is reduced to 9 per cent.

Summary

It is suggested that the RPCF test is a good routine test for treponemal disease, because (*a*) it is technically simple, (*b*) it depends upon an antibody different from reagin, (*c*) it can be readily combined with a WR as a simple routine test, (*d*) it is sensitive and reproducible, and (*e*) it appears to be specific.

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